MICROSPOROGENESIS, MEGASPOROGENESIS, AND EMBRYO SAC DEVELOPMENT AT FIVE PLOIDY LEVELS IN **Digitaria**

By
JOHN W. CARMICHAEL

A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL OF
THE UNIVERSITY OF FLORIDA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA
1970

ACKNOWLEDGMENTS

The writer is grateful to all those who helped and encouraged him throughout his college years. He is especially grateful to his wife, Elaine; his grandparents, the late Mr. and Mrs. J. B. Carmichael; and his parents, Mr. and Mrs. M. W. Carmichael.

Deep appreciation is extended to Dr. S. C. Schank, Associate Professor of Agronomy, for his conscientious guidance, understanding, and encouragement. Sincere thanks are extended to Dr. Mildred Griffith, Professor of Botany; Dr. W. B. Sherman, Assistant Professor of Fruit Crops; and Dr. F. T. Boyd, Professor of Agronomy, for serving as members of the writer's supervisory committee.

The interest and encouragement provided by Dr. A. T. Wallace, Assistant Dean for Research, is greatly appreciated.

The writer greatly values the friendship of Mrs. Glenda Smith and Miss Jane Parker, secretaries in the Agronomy Department, and thanks them for the many favors performed.

TABLE OF CONTENTS

	Page
ACKNOWLEDGMENTS	ii
LIST OF TABLES	v
LIST OF FIGURES	vi
ABSTRACT	x
INTRODUCTION	1
REVIEW OF LITERATURE	3
Nomenclature of the Genus *Digitaria*	3
Cytogenetics of *Digitaria*	4
Chromosome Numbers and Pachytene Morphology	4
Microsporogenesis and Pollen Viability	10
Megasporogenesis and Embryo Sac Development	17
MATERIALS AND METHODS	20
Plant Materials	20
Methods	20
Method of Studying Microsporogenesis	20
Methods of Studying Megasporogenesis and Embryo Sac Development	22
Methods of Studying Pollen Viability	23
Method of Studying Fertilization	25
RESULTS	26
Microsporogenesis and Pollen Viability	26
D. swazilandensis, FL 615	26
D. decumbens, FL 594	26
D. pentzii, FL 640	27
D. pentzii, FL 645	27
Hybrid X125-1	30
D. sp., FL 525	32

Page

Megasporogenesis and Embryo Sac Development 32
Megasporogenesis 32
Embryo Sac Development 34

Fertilization 35

DISCUSSION 77

D. swazilandensis, FL 615 77
D. decumbens, FL 594 77
D. pentzii, FL 640 77
D. pentzii, FL 645 79
Hybrid X125-1 80
D. sp., FL 525 80

SUMMARY 81

LITERATURE CITED 83

BIOGRAPHICAL SKETCH 87

LIST OF TABLES

Table		Page
1	Chromosome numbers reported in *Digitaria*	5
2	Accession and Chromosome numbers of plant materials	21
3	The effect of storage periods of varying duration on *in vitro* pollen germination in *D. pentzii*, FL 645	29
4	The effect of a wide range of gibberellic acid concentrations on *in vitro* pollen germination in *D. pentzii*, FL 645	29
5	The effect of a narrow range of gibberellic acid concentrations on *in vitro* pollen germination in *D. pentzii*, FL 645.	31
6	The effect of manitol and gibberellic acid on *in vitro* pollen germination in *D. pentzii*, FL 645	31
7	Frequency of ovule abortion	36

LIST OF FIGURES

Figure		Page
1	Nine bivalents at metaphase I in a PMC of D. swazilandensis, FL 615. X1500	39
2	Eighteen univalents at anaphase I in a PMC of D. swazilandensis, FL 615. X750	39
3	Two lagging univalents at telophase I in a PMC of D. swazilandensis, FL 615. X1000	40
4	A configuration of 9II + 9I at metaphase I in a PMC of D. decumbens, FL 594. X700	40
5	Twenty-seven univalents at anaphase I in a PMC of D. decumbens, FL 594. X1000	41
6	Three dividing univalents at anaphase I in a PMC of D. decumbens, FL 594. X1200	41
7	A chromosome distribution of 12-15 at telophase I in a PMC of D. decumbens, FL 594. X1200	42
8	Lagging chromosomes at anaphase and telophase II in secondary microsporocytes of D. decumbens, FL 594. X1200	42
9	A configuration of 18II + II at metaphase I in a PMC of D. pentzii, FL 645. X1500	43
10	A configuration of 16II + IIV + II at metaphase I in a PMC of D. pentzii, FL 645. X1200	43
11	Thirty-seven univalents at anaphase I in a PMC of D. pentzii, FL 645. X1500	44
12	A chromosome distribution of 18-18 plus 1 laggard at telophase I in a PMC of D. pentzii, FL 645. X1200	44
13	A divided univalent at telophase I in a PMC of D. pentzii, FL 645. X1200	45

Figure		Page
14	Eight lagging univalents at telophase I in a PMC of D. pentzii, FL 645. X1200	45
15	Excluded chromosomes at metaphase II in secondary microsporocytes of D. pentzii, FL 645. X1200	46
16	Excluded chromosomes at anaphase II in secondary microsporocytes of D. pentzii, FL 645. X1200	46
17	Overlapping chromosomes at metaphase I in a PMC of X125-1. X1200	47
18	Forty-five univalents at anaphase I in a PMC of X125-1. X1200	47
19	Four lagging univalents at anaphase I in a PMC of X125-1. X1200	48
20	Eight excluded chromosomes at prophase II in secondary microsporocytes of X125-1. X1200	48
21	Lagging chromosomes at telophase II in secondary microsporocytes of X125-1. X1000	49
22	Overlapping chromosomes at metaphase I in a PMC of FL 525. X1200	49
23	Late anaphase I in a PMC of D. sp., FL 525. X1200	50
24	Two lagging chromosomes at anaphase I in a PMC of D. sp., FL 525. X1200	50
25	Cells from a single anther of D. sp., FL 525, showing asynchrony of meiosis. X250	51
26	A MMC at prophase I in an ovule of D. sp., FL 525. X750	51
27	An egg cell and 3 antipodals in an embryo sac of D. pentzii, FL 645. X750. Figures 27 and 28 show serial sections of the same embryo sac	52
28	Two synergids, 2 polar nuclei, and 3 antipodals in an embryo sac of D. pentzii, FL 645. X750. Figures 27 and 28 show serial sections of the same embryo sac	53

Figure		Page
29	A MMC with a centrally located nucleus in an ovule of X125-1. X625	54
30	A degenerating MMC in an ovule of X125-1. X625	55
31	A 4 nucleate embryo sac and a hypertrophied nucellar cell in an ovule of D. pentzii, FL 645. X1200	56
32	A persistent megaspore and 3 hypertrophied nucellar cells in an ovule of D. pentzii, FL 645. X1000	57
33	A dyad of secondary megasporocytes in an ovule of D. pentzii, FL 640. X750	58
34	Metaphase II in a secondary megasporocyte in an ovule of D. pentzii, FL 645. X625	59
35	A persistent megaspore, a degenerating megaspore, and a degenerating secondary megasporocyte in an ovule of D. sp., FL 525. X1000	60
36	A hypertrophied nucellar cell, a persistent megaspore, a degenerating megaspore, and a degenerating secondary megasporocyte in an ovule of D. pentzii, FL 645. X750	61
37	A persistent megaspore in an ovule of D. pentzii, FL 645. X750	62
38	All megaspores degenerating in an ovule of D. pentzii, FL 645. X1000	63
39	The functional megaspore in an ovule of D. pentzii, FL 640. X750	64
40	A 2 nucleate embryo sac in an ovule of D. pentzii, FL 645. X750	65
41	An 8 nucleate embryo sac in an ovule from D. pentzii, FL 645. X625	66
42	An egg cell and 2 polar nuclei in an embryo sac of D. swazilandensis, FL 615. X1250	67
43	Two synergids and 1 polar nucleus in an embryo sac of D. swazilandensis, FL 615. X1250	68

Figure		Page
44	A persistent megaspore of D. pentzii, FL 640, extended through the nucellar epidermis and in contact with an inner integument. X1000	69
45	An extra-ovular embryo sac in an ovary of D. pentzii, FL 640. X300	70
46	An egg cell, 1 polar nucleus, and 2 antipodals in an ovule of X125-1. X750. Figures 46, 47, and 48 show serial sections of the same ovule	71
47	Degenerating synergids, 1 polar nucleus, and 2 antipodals in an ovule of X125-1. X750. Figures 46, 47, and 48 show serial sections of the same ovule	72
48	An egg cell in an ovule of X125-1. X750. Figures 46, 47, and 48 show serial sections of the same ovule	73
49	One large nucleus and several antipodals in an embryo sac of X125-1. X750	74
50	An aberrant multicellular structure in an ovule of X125-1. X1000	75
51	An embryo sac of D. pentzii, FL 645, killed 30 minutes after pollination with D. pentzii, FL 640. X300	76

Abstract of Dissertation Presented to the
Graduate Council of the University of Florida in Partial Fulfillment
of the Requirements for the Degree of Doctor of Philosophy

MICROSPOROGENESIS, MEGASPOROGENESIS, AND EMBRYO SAC DEVELOPMENT AT FIVE PLOIDY LEVELS IN Digitaria

By

John W. Carmichael

December, 1970

Chairman: S. C. Schank
Major Department: Agronomy

Microsporogenesis, megasporogenesis, and embryo sac development were studied in six accessions of Digitaria (Gramineae), representing five ploidy levels.

Meiosis was regular in pollen mother cells of a diploid D. swazilandensis (2n=18); slightly irregular in a tetraploid D. pentzii (2n=36), and a hexaploid D. sp. (2n=54); and highly irregular in a triploid D. decumbens (2n=27), an aneuploid D. pentzii (2n=37), and a pentaploid interspecific hybrid, X125-1, (2n=45).

Meiosis in megaspore mother cells produced a linear triad of cells in all accessions. The chalazal member of the triad served as the functional megaspore. Abortion of megaspore mother cells and megaspores was common in triploid D. decumbens and the pentaploid hybrid, X125-1.

Mature embryo sacs were formed in all accessions, and contained one egg cell, two synergids, two polar nuclei, and several antipodals. The percent ovule abortion varied greatly among the six accessions, ranging from 0, in diploid D. swazilandensis, to more than 90, in triploid D. decumbens.

Abnormal embryo sacs were found in three accessions. One of six post-anthesis ovaries from the triploid D. decumbens contained a large

embryo sac with a single nucleus. Forty percent of the mature embryo sacs of the tetraploid D. pentzii extended through the nucellar epidermis and the micropyle into the ovarian locule. One of 41 ovaries from the pentaploid hybrid, X125-1, contained an embryo sac with no egg apparatus, one large nucleus in the position normally occupied by the polar nuclei, and several antipodals. Four X125-1 ovules contained more than the normal complement of gametophytic structures. Three X125-1 ovules contained two egg cells, two to eight polar nuclei, and several antipodals. Another X125-1 ovule contained an embryo sac with one egg cell, three polar nuclei, and several antipodals.

In vitro pollen germination was obtained on a medium of 3.0 percent agar, 12.0 percent sucrose, 0.02 percent boric acid, and 0.02 percent calcium nitrate, using pollen from the aneuploid D. pentzii. The addition of 40 ppm gibberellic acid to the germination medium more than doubled the percent germination.

INTRODUCTION

In recent years, members of the grass genus Digitaria have become widely used as forage throughout tropical and subtropical regions of the western hemisphere. Interest in the genus was initially stimulated by reports of high yields of high quality forage from Digitaria decumbens Stent, or pangolagrass (27). Pangolagrass originated as an introduction from South Africa in 1935, and an estimated half million acres of pangolagrass were being grown in Florida by 1955. Despite the phenomenal success of pangolagrass in Florida and Latin America, it has four undesirable characteristics, namely, complete male and female sterility (42), lack of winter hardiness (36), and susceptibility to pangola stunt virus (16, 39) and sting nematodes (1). Since pangolagrass is sterile, and genetically uniform, its characteristics can not be altered by conventional breeding methods.

Other members of the genus Digitaria have shown considerable potential as forage species. In Puerto Rico, a cultivar of Digitaria valida Stent, known as giant pangola, has received much attention (43). In Florida, a Digitaria species was released to farmers under the common name, slenderstem digitgrass (25).

Sterility and lack of variability in pangolagrass, together with a paucity of Digitaria germ plasm in the western hemisphere, resulted in a plant collection expedition by the United States Department of Agriculture. In 1964, Oakes (28) collected 316 accessions of Digitaria in Africa. The Oakes collection has served as a major source of germ

plasm for a Digitaria breeding program at the University of Florida since that time.

Low pollen fertility and seed set were found to be common among 28 Digitarias selected from the Oakes collection (37). An inverse relationship between seed set and temperature was reported in two accessions of Digitaria milanjiana (Rendel) Stapf (46). The highest rate of seed set was 28.5 percent.

The cause of low seed set in Digitaria valida Stent has been suggested to result from competition between "apomictic nucellar cells" and sexual embryo sacs (30). Brown and Emery (3), however, reported that mature embryo sacs of 8 species of Digitaria, including D. valida, are "normal," i.e., are probably not the products of apospory. In a separate publication, the same authors noted the occurrence of extra-ovular embryo sacs in D. valida (37).

Further information is needed on the mode of reproduction and causes of low seed set in this important genus of grasses. An understanding of microsporogenesis, megasporogenesis, and embryo sac development would be valuable to the breeding program at the University of Florida and aid in clarifying the taxonomy of the genus.

REVIEW OF LITERATURE

Nomenclature of the Genus Digitaria

The name Digitaria was first published in accordance with present rules of botanical nomenclature by Fabricius in 1759 (20). The author of the genus is Heister. Heister, a prelinnean predecessor of Fabricius, applied the name Digitaria to the plant now known as Digitaria sanguinalis (L.) Scop.

The genus Digitaria is large and distributed throughout tropical and subtropical regions of the world. Henrard (20), in his Monograph of the Genus Digitaria, recognized 325 species of Digitaria. Brown (2) estimated the number of species to be about 100. Chippendall (8) recognized 30 species of Digitaria, 26 of which were listed as indigenous to Africa.

Henrard separated the genus Digitaria into 4 subgenera: the Solitaria, Setariopsis, Leptoloma, and Eu-Digitaria. The first 3 subgenera are small, containing only 19 species. The subgenus Eu-Digitaria is partitioned into 32 sections. The largest and most agronomically important section is the Erianthae, with 34 species. According to Henrard, the section includes D. decumbens Stent, D. eriantha Steud., D. milanjiana (Rendel) Stapf, D. pentzii Stent, D. seriata Stapf, D. smutsii Stent, and D. valida Stent. Chippendall suggested Henrard's section, Erianthae, be treated as a single polymorphous species comprising different "races, varieties, forms, or strains." Chippendall,

however, preserved this section, citing a need for further knowledge of the variation which occurs within this taxonomically difficult group.

Cytogenetics of Digitaria

Chromosome Numbers and Pachytene Morphology

Chromosome numbers have been reported for 42 species of Digitaria. The chromosome numbers reported for 42 species, with several counts for some species, are presented in Table 1. Sixty-one of 79 chromosome numbers reported were multiples of 9. The lowest chromosome number found was 18, and occurred in 24 percent of the counts. The basic chromosome number for the genus has been suggested to be x=9 (29, 37, 38).

A wide range of ploidy levels occurs within the genus and within individual species. Polyploidy was found in 60 of the 79 reported chromosome numbers. Eight different chromosome numbers were listed for D. sanguinalis (2, 9, 10, 11). Aneuploidy was reported in 18 of the 60 polyploid counts.

Pangolagrass (D. decumbens) has a triploid chromosome number of 2n=27, and is believed to be a natural interspecific hybrid from parents having 2n=18 and 2n=36 chromosomes. D. pentzii has been suggested as the lower ploidy parent of pangolagrass (38).

Shambulingappa (40) studied pachytene chromosome morphology in 9 diploid accessions of Digitaria. Four types of pachytene chromosome morphology were found. In the first type, centromeres were easily distinguished, generally achromatic, but long in some chromosomes and short and oval in others. All centromeres were flanked by deeply

Table 1. Chromosome numbers reported in Digitaria.

Species	2n	Reported by
D. adscendens (H.B.K.) Henr.	27	Chen and Hsu (7).
" "	54	Chen and Hsu (7), Gould (19), Schank and Decker (37), Tateoka (44).
" "	72	Schank and Decker (37).
D. adusta (Nees) Griseb.	72	Darlington and Wylie (11).
D. brazzae (Franch.) Stapf	18	Moffett and Hurcombe (26).
D. chinensis (Nees) Camus	18	Chen and Hsu (7), Darlington and Wylie (11), Tateoka (44).
D. ciliaris Fig. et DeNot.	54	Hirayoshi and Yasue (21).
D. decumbens Stent	18	Shambulingappa (40).
" "	27	Rotar and Urata (32), Schank (34), Schank and Decker (37), Schank et al. (38), Sheth et al. (42).
" "	30	Burton (4), Sheth[a].
D. diagonalis (Nees) Stapf	36	de Wet and Anderson (15).
D. didactyla Willd.	18	Darlington and Wylie (11), Pritchard and Gould (29), Schank and Decker (37).
" "	36	Pritchard and Gould (29).
D. eriantha Steud.	18	Moffett and Hurcombe (26).
D. eriantha Steud.	40	de Wet (12).

Table 1. Continued.

Species	2n	Reported by
D. exilis (Kippist) Stapf	54	Hunter (22).
D. filiformis (L.) Koel.	36	Brown (2).
D. gazensis Rendel	18	Moffett and Hurcombe (26).
D. griffithii (Arn.) Henr.	54	Raman et al. (31).
D. henryi Rendel	36	Chen and Hsu (7).
D. horizontalis Willd.	30	Darlington and Wylie (11).
" "	36	Darlington and Wylie (11).
D. iburua Stapf	36	Schank (34).
D. insularis (L.) Mez.	36	Tan[b].
D. ischaemum (Schreb.) Schreb.	34	Darlington and Wylie (11).
" "	36	Brown (2).
D. leptalea Ohwi	36	Chen and Hsu (7).
D. littoralis Salisb.	18	de Wet and Anderson (15).
D. longiflora (Retz.) Pers.	18	Chen and Hsu (7), de Wet and Anderson (15).
" "	34	Darlington and Wylie (11).
D. macroglosa Henr.	18	Shambulingappa (40).
" "	36	Darlington and Wylie (11).
D. magna (Honda) Tuyama	36	Chen and Hsu (7).
D. marginata Link	18	Chandrasekharan (6).
" "	36	Raman et al. (31).
" "	54	Raman et al. (31).
" "	72	Darlington and Wylie (11).

Table 1. Continued.

Species	2n	Reported by
D. microbachne (Presl) Henr.	72	Chen and Hsu (7).
D. milanjiana (Rendel) Stapf	18	Moffett and Hurcombe (26).
" "	34	Schank and Decker (37).
" "	36	Tan[b], Schank and Decker (37).
D. monodactyla (Nees) Stapf	36	de Wet (14).
D. natalensis Stent	18	Schank (34).
D. pentzii Stent	18	Pritchard and Gould (29), Schank and Decker (35), Schank et al. (38).
" "	35	Pritchard and Gould (29).
" "	36	Burton (4), Schank and Decker (36).
" "	54	Moffett and Hurcombe (26).
D. polevansii Stent	34	Burton (4).
" "	36	Shambulingappa (41).
" "	45	Tan[b].
D. praecox (Walt.) Willd.	18	Hirayoshi and Yasue (21).
D. sanguinalis (L.) Scop.	28	Church (9).
" "	34	Darlington and Wylie (11), Schank (34).
" "	36	Brown (2), de Wet and Anderson (15), Gould (18).
" "	40	Brown (2).

Table 1. Continued.

Species	2n	Reported by
D. sanguinalis (L.) Scop.	44	Brown (2).
" "	45	Brown (2).
" "	48	Brown (2).
" "	54	Covas and Schnack (10).
D. seriata Stapf	36	de Wet and Anderson (15).
D. sericea (Honda) Honda	54	Chen and Hsu (7).
D. setivalva Stent	36	Schank and Decker (37).
D. shimadana Ohwi	36	Chen and Hsu (7).
D. smutsii Stent	18	Darlington and Wylie (11), Tan[b], Schank and Decker (37).
D. sp.	18	Schank (34), Shambulingappa (40).
" "	36	Burton (4), McCaleb and Hodges (25).
" "	54	Tan[b], Schank and Decker (37).
D. swazilandensis Stent	18	Moffett and Hurcombe (26), Schank and Decker (37), Shambulingappa (40).
" "	27	Schank and Decker (37).
D. ternata (Hochst.) Stapf	30	de Wet (13).
D. tricholaenoides Stapf	18	de Wet and Anderson (15).
" "	30	de Wet (14).
D. valida Stent	18	Shambulingappa (40).
" "	24	Young and Crocker (50).

Table 1. Continued.

Species	2n	Reported by
D. valida Stent	30	de Wet (12).
" "	36	de Wet and Anderson (15), Schank and Decker (37).
" "	42	Sotomayor-Rios et al. (43).
" "	45	Schank and Decker (37).
D. violascens Link	36	Chen and Hsu (7).

[a]Sheth, A. A. 1955. Sterility problems in Digitaria decumbens Stent. M.S.A. Thesis. Univ. of Florida. Gainesville, Florida. 74 p.

[b]Tan, N. V. 1964. Cytogenetics and hybridization of six Digitaria species. M.S.A. Thesis. Univ. of Florida. Gainesville, Florida. 62 p.

staining segments followed by a lightly staining region. One or 2 bivalents possessed knobs, and nucleolar organizers were terminal. In the second type, the position of centromeres could not be easily distinguished, and knobs were absent. Nucleolar organizers were terminal, but followed by deeply staining chromomeres. In the third type, the centromeric position could not be determined. All nine bivalents stained uniformly, but seven lightly and two deeply. Nucleolar organizers were terminal and not followed by deeply staining chromomeres. In the fourth type, centromeres appeared as short constrictions in some bivalents, and could not be resolved in others. The amount of heterochromatin in individual chromosomes varied from almost none to 2/3 of a chromosome, and nucleolar organizers were terminal.

Microsporogenesis and Pollen Viability

Meiotic chromosome behavior during microsporogenesis has been described for several species of Digitaria. In these accounts, meiosis has been termed "regular" if: 1. No univalent or multivalent chromosomes were found at late prophase or first metaphase. 2. No lagging univalent or bivalent chromosomes were observed at anaphase of either meiotic division. 3. No chromosome bridges or fragments were observed at anaphase or telophase of either meiotic division. 4. No micronuclei were observed at any stage during the two meiotic divisions. If the presence of any of these phenomena was noted meiosis was said to be "irregular."

Diploid accessions

Microsporogenesis was described as regular in diploid accessions of D. natalensis, D. pentzii, and D. praecox (34, 38).

Tan[1] described meiosis as regular in 2 diploid accessions of D. smutsii.

Schank and Decker (37) studied pollen viability in 6 diploid accessions of Digitaria representing 5 species. Pollen viability was estimated by staining with analine blue in lactophenol. Pollen viability was found to be very low in two diploid accessions of D. pentzii. Only 0.4 and 2.8 percent of the pollen of these accessions appeared healthy. A diploid accession of D. gazensis produced only 0.9 percent stainable pollen. Diploid accessions of D. milanjiana, D. smutsii, and D. swazilandensis produced much higher percentages of viable pollen. These species produced 43.7, 38.5, and 66.2 percent stainable pollen, respectively.

Triploid accessions

Meiosis has been studied in pollen mother cells of two triploid Digitarias. Chen and Hsu (7) described meiosis in a triploid accession of D. adscendens as very irregular. Eight or 9 univalent chromosomes were found in most pollen mother cells at diakinesis and metaphase I. Lagging chromosomes were common at anaphase I. Chromosomes excluded from daughter nuclei of the first division were excluded from the metaphase plate of the second division.

Microsporogenesis was studied in triploid pangolagrass (D. decumbens) by Sheth et al. (42) and Schank et al. (38). Schank et al. described meiosis as irregular, with up to 9 lagging univalent chromosomes at anaphase I. Sheth et al. described meiosis in pollen mother cells as irregular, with from 1 to 7 univalents at metaphase I. At

---

[1] Ibid., p. 9.

anaphase I, 86.6 percent of the pollen mother cells examined contained 1 or more lagging chromosomes. In 112 cells at telophase I, 56 contained 1 or more chromosomes excluded from the daughter nuclei. Excluded chromosomes were observed in 10.9 percent of the cells at metaphase II, and in 34.8 percent of the anaphase II cells. Excluded chromosomes were found in 29.5 percent of the tetrads, and occasional pentads were formed. The small accessory cell of the pentads contained 1 or 2 chromosomes. Both authors estimated pollen viability in pangolagrass at 0.4 percent.

Tetraploid accessions

Descriptions of meiotic chromosome behavior have been given for a number of tetraploid Digitarias. Most tetraploids have been described as having consistent synapsis with only bivalents present at diakinesis and metaphase I, and regular disjunction at anaphase I.

Meiosis was reported to be regular in 3 tetraploid accessions of D. valida (47). Pollen stainability was determined for 2 of the accessions, yielding values of 14 and 94 percent. Attempts to germinate pollen from tetraploid D. valida accessions on several types of artificial media were unsuccessful.

Microsporogenesis in a tetraploid accession of D. ischaemum was studied by Hirayoshi and Yasue (21). Meiotic chromosome behavior in pollen mother cells was described as regular.

Tan[1] found meiotic chromosome behavior in pollen mother cells of 5 tetraploid Digitarias to be regular. The tetraploid plants investigated were D. insularis, D. milanjiana, D. sp. UF 155, a plant described

---

[1] Ibid., p. 9.

as probably derived by self pollination or apomixis from an unidentified Digitaria with 36 chromosomes, and a plant described as probably derived by self pollination or apomixis from a tetraploid accession of D. insularis. Pollen stainability in the 5 accessions ranged from 26.6 to 88.7 percent.

The same author reported irregular chromosome behavior in a tetraploid Digitaria. This accession was a hybrid from a cross using a tetraploid D. milanjiana and a pentaploid D. polevansii as parents. In this hybrid, 17 bivalent and 2 univalent chromosomes were observed at metaphase I. Lagging chromosomes were seen at anaphase and telophase I. Lagging univalent chromosomes moved toward the spindle poles, undivided, at random to each other, or divided with the halves of each laggard moving toward opposite poles. Up to 8 lagging chromosomes were observed in a single cell at telophase I. Micronuclei were frequently observed at the tetrad stage. Pollen stainability was 33.8 percent.

Shambulingappa (41) described a type of male sterility in a tetraploid accession of D. polevansii characterized by extremely irregular chromosome behavior. In a few cells at diakinesis and metaphase I, meiosis appeared regular, with the formation of 18 bivalents. In most cells, however, meiosis was irregular at all stages. At pachytene, pairing was often loose. Asynchrony of bivalent condensation was prevalent in some cells at diplotene, some bivalents appeared as small condensed bodies while others remained long and thread-like. In many pollen mother cells, 3 or 4 nuclear bodies were found at diplotene. The occurrence of a quadrivalent was noted in a few cells at diakinesis. Diffused chromatin bodies were seen on the equatorial plate at metaphase I. Lagging chromosomes, chromatin bridges and restitution nuclei were

found in cells reaching anaphase I. Regular 18 - 18 disjunction was noted in a small percentage of cells at anaphase I. Metaphase and anaphase II stages were seen. Fully developed anthers were generally found to contain single cells with 1 to 4 nuclei and micronuclei. Pollen sterility was estimated at 85 to 90 percent in this accession.

Pollen stainability was described by Schank and Decker (37) in 15 tetraploid accessions of Digitaria representing 4 species. The stainability of 5 tetraploid accessions of D. milanjiana was 50.5, 58.1, 74.9, 82.4, and 89.1 percent. Three accessions of D. pentzii produced 58.1, 68.2, and 83.9 percent stainable pollen. Four tetraploid accessions of D. setivalva gave 2.6, 6.0, 69.2, and 78.5 percent stainable pollen. Three accessions of D. velida produced 16.0, 54.8, and 70.8 percent stainable pollen.

Pentaploid accessions

Irregularities in meiotic chromosome behavior were reported for all pentaploid Digitarias studied. Tan[1] found an introduced accession of D. polevansii to be a natural pentaploid with highly irregular meiosis. Eighteen bivalent and 9 univalent chromosomes were observed in 15 cells at metaphase I. In 4 other cells at this stage, 20 bivalent and 5 univalent chromosomes were found. At telophase I, up to 9 lagging chromosomes were observed. Lagging chromosomes were also observed at anaphase II. Laggards were often excluded from the daughter nuclei resulting from the two meiotic divisions. However, 77.6 percent of the pollen produced by this accession was completely filled by cytoplasm

---

[1] Ibid., p. 9.

and stainable. One seed was found in 498 spikelets, establishing the partial fertility of this accession.

Meiosis was reported as highly irregular in pollen mother cells of 5 pentaploid interspecific hybrids from the cross D. ciliaris (6x) x D. ischaemum (4x) by Yasue (49). Pairing configurations varied from 8 bivalent plus 29 univalent chromosomes to no bivalent and 45 univalent chromosomes at metaphase I. The most common configuration was 3 bivalent plus 39 univalent chromosomes. The average percent pollen stainability for the 5 hybrids was 0.26.

Schank and Decker (37) reported 34.0 percent pollen stainability in a pentaploid accession of D. valida.

Hexaploid accessions

Regular meiotic chromosome pairing and disjunction were reported for each of 3 hexaploid Digitarias studied by Tan[1]. The hexaploids were D. ciliaris, D. sp. UF 187, and a plant described as probably derived by self pollination or apomixis from a hexaploid D. ciliaris. Pollen stainability was reported as 72.6 percent in the D. sp., 78.1 percent in the plant obtained from a D. ciliaris seed, and was not reported for the D. ciliaris accession.

The percent pollen stainability was 84.5 in an unidentified hexaploid accession of Digitaria studied by Schank and Decker (37).

Aneuploid accessions

Aneuploidy and irregular meiotic chromosome behavior were reported in an accession of D. sanguinalis. Church (9) reported a chromosome number of 2n=28 for this accession, with 14 ring bivalents at diakinesis.

---

[1] Ibid., p. 9.

Cytomixis resulted in masses of chromatin in the cytoplasm of pollen mother cells. Lagging bivalents were reported as frequent during the first division of meiosis. The second meiotic division was described as normal, except in a few cells where a multinucleate condition was found.

A chromosome number of 2n=28 was reported for a plant described as morphologically intermediate between D. adscendens and D. sanguinalis (19). Meiotic chromosome behavior in pollen mother cells of this aneuploid was described as irregular, with multivalents consistently present at prophase and metaphase I. Two to 4 lagging chromosomes were frequently observed at early telophase I.

Descriptions of microsporogenesis have been given for 4 aneuploid accessions of D. valida. Young and Crocker (50) described meiosis in an accession of D. valida with 24 chromosomes. Metaphase plates of 8 bivalent and 8 univalent chromosomes were reported. Univalents migrated at random to either pole at anaphase I. Micronuclei were found at the tetrad stage. Pollen stainability was 16 percent, but attempts to germinate pollen in sucrose solutions were unsuccessful.

Meiotic irregularities were found in 2 aneuploid accessions of D. valida (47). In 1 accession, 18 bivalent chromosomes and from 1 to 4 univalent "accessary" chromosomes were found at metaphase I. In the other D. valida accession, 18 bivalent and 1 or 2 univalent chromosomes, or 19 bivalent chromosomes were found at metaphase I. Pollen stainability was 77 percent in the former, and 70 percent in the latter accession.

An aneuploid accession of D. valida with 42 chromosomes was found by Sotomayor-Rios et al. (43). Meiosis in pollen mother cells was

highly irregular. Univalents, bivalents, trivalents and quadrivalents were observed at prophase and metaphase I. The mean number of bivalents at metaphase I was 14.5, with a maximum of 18 per cell. The mean number of univalents per cell was 4, and the maximum 8, at metaphase I. The highest degree of multivalent association in a single cell was 3 trivalents and 2 quadrivalents. Lagging univalent chromosomes were present in all anaphase I cells. A mean of 3.8 laggards per cell was reported. Lagging univalents divided equationally at anaphase I, but the half univalents often failed to be included in the daughter nuclei. Anaphase I distribution was often unequal. The second meiotic division was also highly irregular. An average of 4.6 micronuclei per tetrad was reported. Only 2.3 percent of the pollen grains stained completely, and these were variable in size.

Megasporogenesis and Embryo Sac Development

An examination of 196 sectioned ovaries of triploid pangolagrass (D. decumbens) revealed that megasporogenesis did not proceed beyond the leptotene stage of the first meiotic prophase (42). Megaspore mother cells degenerated shortly after entering prophase I, leaving a line of densely staining material extending from the center of the nucellus toward the micropyle. No embryo sacs were observed.

Six species of Digitaria have been found to form embryo sacs which were 8 nucleate, 7 celled, and presumably haploid (3). The species used were Digitaria argyrograpta (Nees) Stapf, D. natalensis, D. seriata, D. setivalva, D. smutsii, and D. valida.

Female sterility in giant pangola (D. valida, 2n=42) was attributed to degeneration of megaspores by Sotomayor-Rios et al. (43). Megaspore mother cells underwent meiotic divisions, forming an unreported number

of megaspores. One megaspore persisted longer than others, but degenerated before undergoing mitosis. No embryo sacs were found.

Purcell (30) examined ovaries from two accessions of D. valida. Previous studies of pollen mother cells of the accessions revealed one to be tetraploid (2n=36) and the other aneuploid (2n=37-40). Megasporogenesis and embryo sac development were similar in the two accessions. Mature ovaries were said to contain a single anatropous ovule. Within the ovule, two meiotic divisions of the megaspore mother cell produced a linear tetrad of megaspores. The three micropylar megaspores were seen to degenerate, leaving the chalazal megaspore functional in embryo sac development. A mitotic nuclear division occurred withing the functional megaspore, forming a 2 nucleate embryo sac. Two further mitotic divisions gave rise to 8 nucleate embryo sacs. Three of the 8 nuclei were included in the egg apparatus, consisting of the egg cell and two synergids. Another pair of nuclei functioned as the polar nuclei. The remaining 3 nuclei proliferated and were included in 7 or 8 antipodal cells.

According to Purcell 50 to 60 percent of the ovaries examined showed a "strong tendency to apomixis." This statement was based on the observation of enlarged nucellar cells adjacent to developing sexual embryo sacs. Enlarged nucellar cells occurred in 50 to 60 percent of the ovaries examined, and were usually located near the antipodals of the sexual embryo sac. One 4 nucleate "apomictic sac" was observed.

The presence of enlarged nucellar cells in an ovule was said to delay or arrest the development of the sexual embryo sac in that ovule. It was suggested that low seed set is probably due to competition between enlarging nucellar cells and developing sexual embryo sacs.

A peculiar type of embryo sac development was noted by Emery and Brown (17) in 1 of 55 D. valida ovaries examined. In this ovary, the embryo sac had grown through the chalazal end of the ovule into the ovary wall. The egg apparatus protruded outside the ovule into a cavity enclosed by the ovary wall.

MATERIALS AND METHODS

Plant Materials

Five accessions varying from diploid to hexaploid were selected from the Digitaria introduction nursery at the University of Florida. The accessions selected and their plant introduction and chromosome numbers appear in Table 2.

A sixth accession studied, X125-1, was a pentaploid interspecific hybrid produced by Dr. S. C. Schank of the Agronomy Department at the University of Florida. The female parent of this hybrid was D. pentzii, FL 640, and the male parent D. sp., FL 525.

The accessions bearing U.S.D.A. plant introduction numbers 299606 and 299837 were listed by Schank and Decker (37) as D. decumbens and D. swazilandensis, respectively, in agreement with field identifications made at the time of collection. A more recent report by Decker (unpublished) identified P.I. 299606 as D. pentzii and P.I. 299837 as D. decumbens. The latter identifications were used in this report.

Herbarium specimens of each accession were filed with the Florida Agricultural Experiment Station Herbarium at Gainesville, Florida.

Methods

Method of Studying Microsporogenesis

Meiotic chromosome behavior during microsporogenesis was studied by the aceto-orcein squash method. Entire inflorescences were fixed

Table 2. Accession and Chromosome numbers of plant materials.

Species	U.S.D.A. P.I. No.	Univ. of Fla. P.I. No.	Reported Chromosome Number (37)
D. swazilandensis	299840	FL 615	2n = 18
D. decumbens	299837	FL 594	2n = 27
D. pentzii	299748	FL 640	2n = 36
D. pentzii	299606	FL 645	2n = 36 or 37
D. sp.	299892	FL 525	2n = 54

and stored in a modified Carnoy's solution consisting of 10 parts absolute ethanol, 3 parts glacial acetic acid, and 3 parts chloroform. Single anthers were removed from fixed spikelets, cut into short segments, and squashed in a drop of aceto-orcein on a microscope slide. A cover glass was applied, the specimen heated and then flattened by applying pressure to the cover glass. Photographs of meiotic chromosomes were made with Kodak High Contrast Process Ortho, 4 X 5 inch sheet film.

Methods of Studying Megasporogenesis and Embryo Sac Development

Megasporogenesis and embryo sac development were studied in ovaries from inflorescences killed and fixed in Allen-Bouin Type II solution, prepared according to Sass (33). Fixed inflorescences were washed in water, dehydrated in an ethanol series, and stored in 70 percent ethanol. Ovaries were removed from the spikelets, cleared in a tertiary butanol series, and embedded in Paraplast. Ovaries from spikelets containing anthers with pollen mother cells undergoing meiosis were sectioned at a thickness of 10 microns. More mature ovaries were sectioned at a thickness of 12 microns. Sections were mounted on slides using Haupt's adhesive (33).

Several staining schedules were used on sectioned ovaries. The most effective schedule was a pontacyl violet 6R - orange G schedule, not previously described in the literature. The schedule is as follows:

1. Bring slides to water.
2. Stain 2-3 minutes in 1 percent pontacyl violet 6R in 45 percent acetic acid.
3. Dip in 35 percent ethanol for 10 seconds.

4. Dip in 50 percent ethanol for 10 seconds.
5. Dip in 70 percent ethanol for 10 seconds.
6. Submerge in 95 percent ethanol for 1 minute.
7. Submerge in absolute ethanol for 1 minute.
8. Counterstain in 0.25 percent orange G in a mixture of 2 parts clove oil, 1 part absolute ethanol, and 1 part xylene.
9. Rinse for 10 seconds in a mixture of 2 parts clove oil, 1 part absolute ethanol, and 1 part xylene.
10. Rinse for 10 seconds in a mixture of 1 part clove oil, 1 part absolute ethanol, and 1 part xylene.
11. Submerge in a 1:1 mixture of absolute ethanol and xylene for 3 minutes.
12. Submerge in xylene for 5 minutes.
13. Submerge in xylene for 5 minutes.
14. Apply cover glass with permount.

Other schedules used with moderate success were Safrain O - fast green (24), haematoxylin - orange G (23), pontacyl violet 6R - luxol fast yellow TN (5), and pontacyl blue black SX - luxol fast yellow TN (48).

Photographs of sectioned ovaries were made using Kodak Panatomic X, 4 X 5 inch sheet film.

Methods of Studying Pollen Viability

Pollen stainability

Pollen viability was estimated in all accessions by determining the percent pollen stainability in a 0.1 percent solution of aniline blue in lactophenol. Only grains completely filled with cytoplasm and

fully stained were scored as viable. Observations were made at a magnification of 150 X.

In vitro pollen germination

The second method of estimating pollen viability was to determine the percent germination in vitro. This method was used only on pollen of D. pentzii, FL 645.

Flowering culms were cut from field grown plants, their cut ends submerged in water, and brought into the laboratory the afternoon before pollen was collected. Spikelets were excised from the racemes when their anthers were approximately half exserted and prior to dehiscence. Immediately following excision, the spikelets were placed in small dry cups, over warm water, in petri dishes. The cups were made from short segments of plastic drinking straws attached at one end to a piece of plastic pot label, and measured approximately 0.5 cm. in diameter and depth. The spikelets were stored over water for 2 to 8 hours. After storage, pollen was shaken from the anthers onto the germination medium contained in petri dishes. Each dish received 10 ml. of medium and the pollen from a single spikelet. Replicate dishes were prepared for each treatment. Following transfer of pollen to the germination medium, a minimum of 3 hours incubation was allowed before the percentage of germination was scored. Scoring the percent of germination was accomplished by counting the number of germinated grains in a sample of 100. Scoring was done at a magnification of 150 X.

A basal germination medium was used, consisting of 3.0 percent agar, 12.0 percent sucrose, 0.02 percent $Ca(NO_3)_2 \cdot 4\ H_2O$, and 0.02 percent boric acid. Additions to the basal medium consisted of various concentrations of 90 percent pure gibberellic acid, obtained from Eastman

Organic Chemical Company, and various concentrations of manitol. These additions were made prior to autoclaving at 121° C. for 15 minutes.

The temperature in the laboratory varied between 25° C. and 27° C. during the storage and incubation periods.

Under the experimental conditions used, the time of anther exsertion and pollen collection varied from day-to-day, occurring in each case between 11:30 p.m. and 4:30 a.m. The time from the beginning of anther exsertion to half exsertion was approximately 5 minutes.

Method of Studying Fertilization

Fertilization was studied in pistils of spikelets excised from inflorescences and emasculated at the time the anthers and stigmas were approximately half exserted. Following excision, spikelets were placed on 1.0 percent agar in petri dishes. Pollen used for pollination was collected as described for *in vitro* pollen germination studies, and applied by shaking it from the small storage cups directly onto the stigmas. Spikelets were incubated at room temperature for 15, 30, 45, or 60 minutes following pollination. After incubation, spikelets were killed, fixed and dehydrated. Pistils were removed from the spikelets, embedded, sectioned, stained and photographed by the same procedures used to study megasporogenesis and embryo sac development.

RESULTS

Microsporogenesis and Pollen Viability

D. swazilandensis, FL 615

Microsporogenesis was regular in D. swazilandensis, FL 615. Nine bivalents were consistently observed at metaphase I, Figure 1. Regular disjunction and a diploid number of 2n = 18 chromosomes were seen at anaphase I, Figure 2. Two lagging univalent chromosomes were found in 1 of 221 cells at anaphase and telophase I, Figure 3. The second meiotic division was regular in all cells observed. No micronuclei were found in 100 microspores examined, and pollen stainability was 78.0 percent in a sample of 200 pollen grains.

D. decumbens, FL 594

Meiosis was highly irregular in D. decumbens, FL 594. Most metaphase I configurations were difficult to analyze due to overlapping of bivalents and univalents. Well-spread metaphase chromosomes were seen in 3 cells, each contained 9 bivalents and 9 univalents, Figure 4. Twenty-seven univalents were observed at anaphase I, Figure 5. The mean number of laggards was 3.08 at anaphase and telophase I. Among 37 cells at anaphase or telophase I, 3 had no laggards and 1 had 9 laggards. Lagging univalents either divided or migrated undivided toward the spindle poles at anaphase I. Twenty-four univalents were moving toward the poles undivided, 2 univalents had divided and 1 was dividing in the cell shown in Figure 6. A distribution of 12 - 15 was found in one cell

at telophase I, Figure 7. Lagging chromosomes were observed at anaphase II, Figure 8, and micronuclei were frequent in microspores. Pollen stainability was 11.5 percent in a sample of 200 pollen grains. Most pollen grains were severely shrunken and devoid of cytoplasm.

D. pentzii, FL 640

Irregularities in meiosis were noted in a few cells at all stages in D. pentzii, FL 640. Metaphase I configurations were analyzable in only 2 cells, both showed 18 bivalents. Lagging univalents were found in 24 of 182 cells at anaphase or telophase I, with a maximum of 4 laggards in 1 cell. Lagging chromosomes were less frequent at anaphase and telophase II, occurring in only 2 of 86 cells at these stages. Micronuclei were present in 4 of 100 microspores examined, and pollen stainability was 68.0 percent.

D. pentzii, FL 645

Aneuploidy was found in D. pentzii, FL 645. The chromosomes of this accession stained more intensely and were spread more satisfactorily by squashing than those of other accessions, making analysis of metaphase I configurations possible. A pairing configuration of 18II + II was found in 19 of 40 cells at metaphase I, Figure 9. A configuration of 16II + IIV + II occurred in the remaining 21 cells at this stage, Figure 10. A chromosome number of 2n = 37 was clearly established at anaphase I, Figure 11. Eighty-one of 139 cells at anaphase or telophase I contained a single lagging univalent, Figure 12. This single univalent often divided at late anaphase or telophase I, Figure 13. A cell with 8 lagging univalents is shown in Figure 14, and the highest number of laggards in a single cell was 9. The mean number of lagging univalents

at anaphase and telophase I was 1.43 in 139 cells. Lagging chromosomes were often excluded from the daughter nuclei formed by the first meiotic division, and appeared scattered in the cytoplasm of cells undergoing the second meiotic division, Figures 15 and 16. Micronuclei were common in microspores. Pollen stainability was 82.0 percent in a sample of 200 pollen grains.

In vitro pollen germination tests verified the viability of a moderate percentage of FL 645 pollen, but gave lower estimates of viability than were obtained by staining with analine blue.

The effect of storage periods of varying duration on pollen viability was determined by in vitro germination. Storage periods of 2, 4, 6, and 8 hours were used. Following storage, pollen was transferred to basal medium and allowed to germinate. The percent germination for each of ten replicate pollen samples for each storage period are shown in Table 3. The data indicated that percent pollen germination increased with longer storage periods. The percent germination of pollen stored two hours was significantly lower than when stored six or eight hours, and pollen stored four hours germinated at a significantly lower percentage than when stored eight hours, $P = 0.05$.

The effect of a wide range of gibberellic acid concentrations on the percent germination was studied in a second in vitro pollen viability test. Pollen was stored for 6 hours prior to being transferred to the various germination media. The concentrations of gibberellic acid and percent germination for each of 13 replicate samples are shown in Table 4. The data indicated that 50 ppm gibberellic acid produced an increase in the percent pollen germination, while at concentrations of 100 or 150 ppm germination was inhibited. The percent germination differed significantly at all levels of gibberellic acid, $P = 0.05$.

Table 3. The effect of storage periods of varying duration on *in vitro* pollen germination in *D. pentzii*, FL 645.

Storage Time	Percent Germination										Mean*
2 hr.	11	31	26	27	15	16	15	19	10	24	19.4 a
4 hr.	17	35	22	36	19	25	28	24	25	27	25.8 b
6 hr.	25	22	35	41	23	22	21	28	30	24	27.1 bc
8 hr.	28	35	31	27	33	27	23	21	31	31	28.7 c

*Means followed by the same letter were not significantly different, P = 0.05.

Table 4. The effect of a wide range of gibberellic acid concentrations on *in vitro* pollen germination in *D. pentzii*, FL 645.

G.A. Conc.	Percent Germination													Mean*
0 ppm	14	10	14	7	11	9	7	13	14	6	11	11	15	10.92 a
50 ppm	20	24	20	15	19	11	14	16	18	14	16	15	18	16.92 b
100 ppm	19	14	9	7	10	10	8	7	3	14	9	7	8	9.62 c
150 ppm	2	3	11	8	2	2	11	9	6	8	12	6	2	6.31 d

*Means followed by the same letter were not significantly different, P = 0.05.

A narrower range of gibberellic acid concentrations was employed to determine a level more optimal in the promotion of germination. The concentrations of gibberellic acid and the percent germination obtained for each of 11 replicate pollen samples are shown in Table 5. The percent germination with 40 ppm gibberellic acid was greater than at all other levels of gibberellic acid used, and was more than twice that obtained without gibberellic acid, $P = 0.05$.

An attempt was made to prevent pollen grain bursting which occurred at a high incidence in previous tests. Manitol, a non-metabolizable sugar, was added to the germination medium as an osmoticum. The concentrations of manitol and gibberellic acid and the percent germination obtained are shown in Table 6. The addition of manitol resulted in complete inhibition of pollen germination, while bursting continued to occur. In the treatments where manitol was not used, a significant and greater than one-fold increase in percent germination occurred when 40 ppm gibberellic acid was present, $P = 0.01$.

Hybrid X125-1

Microsporogenesis was highly irregular in the hybrid X125-1. Metaphase I configurations were not analyzable due to tight clumping of chromosomes, Figure 17. A chromosome count of $2n = 45$ was obtained at anaphase I, Figure 18. Lagging chromosomes were numerous at anaphase and telophase I, Figure 19, with one or more present in all 169 cells observed at these stages. The mean number of lagging univalents was 2.96 and the maximum 9 at anaphase and telophase I. Excluded chromosomes remained scattered in the cytoplasm during prophase II, Figure 20. The mean number of excluded chromosomes was 1.64 in 194 cells at prophase II. Lagging chromosomes were also observed during the second meiotic division, Figure 21. Pollen stainability was 12.0 percent.

Table 5. The effect of a narrow range of gibberellic acid concentrations on *in vitro* pollen germination in *D. pentzii*, FL 645.

G.A. Conc.	Percent Germination											Mean*
0 ppm	1	2	6	4	5	2	10	5	9	4	9	5.18 a
20 ppm	3	7	7	4	8	1	7	9	11	6	6	6.27 a
40 ppm	13	7	8	10	18	18	5	8	18	16	15	12.36 b
60 ppm	8	7	11	4	4	7	6	4	8	4	11	6.73 a
80 ppm	2	2	6	2	2	3	6	2	4	4	5	3.45 a

*Means followed by the same letter were not significantly different, P = 0.05.

Table 6. The effect of manitol and gibberellic acid on *in vitro* pollen germination in *D. pentzii*, FL 645.

Conc.	Percent Germination									Mean
0 ppm G.A. 0% Manitol	9	12	10	7	15	17	15	28	9	13.56
40 ppm G.A. 0% Manitol	24	28	20	26	31	36	32	29	29	28.33
40 ppm G.A. 5% Manitol	0	0	0	0	0	0	0	0	0	0.00
40 ppm G.A. 10% Manitol	0	0	0	0	0	0	0	0	0	0.00
40 ppm G.A. 15% Manitol	0	0	0	0	0	0	0	0	0	0.00

D. sp., FL 525

Microsporogenesis in the hexaploid FL 525 was highly irregular. Metaphase I configurations were not analyzable due to overlapping of chromosomes, Figure 22. Metaphase and anaphase I chromosomes appeared only partially condensed and were often attached to each other in non-homologous associations, Figure 23. Lagging chromosomes were found in 52 of 150 cells at anaphase or telophase I, Figure 24, with a mean of 0.59 laggards per cell. Excluded chromosomes or micronuclei were found at telophase of both meiotic divisions. Asynchrony of the meiotic divisions occurred in some anthers. A group of cells from a single anther are shown in Figure 25. The darkest cells were pollen grains, and the more lightly stained and larger cells were pollen mother cells at various stages of the meiotic divisions. Pollen stainability was 76.0 percent in a sample of 200 pollen grains.

Megasporogenesis and Embryo Sac Development

Megasporogenesis

Cytological events leading to the formation of megaspores were similar in all accessions. Digitaria ovaries contained a single ovule oriented in a nearly horizontal position during early stages of development, Figure 26, and a hemianatropous position at anthesis, Figures 27 and 28. Two integuments were present, the inner being 2 cells thick and completely enclosing the ovule at anthesis, and the outer 3 - 4 cells thick and approximately half enclosing the ovule at anthesis.

A single hypodermal cell of the nucellus functioned as the megaspore mother cell. Megaspore mother cells became greatly elongated, occupying almost the entire length of the nucellus. A large nucleus

with a conspicuous nucleolus was found in megaspore mother cells during most of the first meiotic prophase, Figure 26. The megaspore mother cell nucleus was usually located at the micropylar end of the cell, except in a few ovaries of FL 640 and the hybrid X125-1, where it occasionally assumed a median or chalazal position, Figure 29. Megaspore mother cells of the triploid FL 594 and the pentaploid hybrid X125-1 often degenerated prior to meiosis, Figure 30.

Nucellar cells at the chalazal end of the megaspore mother cell were often conspicuous due to nuclear enlargement and densely stained cytoplasm. These hypertrophied nucellar cells remained conspicuous throughout megasporogenesis and persisted as late as the 4 nucleate stage in the development of sexual embryo sacs, Figure 31, but did not undergo divisions or function in embryo sac development. Most often a single nucellar cell was hypertrophied, Figure 26, but as many as three were seen in some ovaries, Figure 32.

Chromosome numbers and configurations could not be determined in the few nuclear divisions observed in megaspore mother cells. The first nuclear division produced two secondary megasporocytes. The micropylar megasporocyte often degenerated shortly after formation, Figure 33, and did not undergo a second division. Chalazal secondary megasporocytes divided, Figure 34, and produced 2 megaspores. A linear triad of cells resulted from the two meiotic division, Figure 35. The micropylar member of the triad was a secondary megasporocyte and the middle and chalazal members were megaspores. Occasionally a linear tetrad of cells was found following megasporogenesis, Figure 36. The micropylar member of the tetrad was a secondary megasporocyte, the two cells in the middle were megaspores, and the chalazal member was a hypertrophied nucellar cell.

The chalazal megaspore persisted and enlarged while the micropylar megaspore and secondary megasporocyte degenerated, leaving a line of densely stained material extending from the persistent megaspore toward the micropyle, Figure 37. Degeneration of all megaspores occurred in most ovaries of FL 594 and to some extent in all other accessions, Figure 38.

Embryo Sac Development

Differences were noted in the cytological events leading to the formation of embryo sacs among the 6 accessions. A generalized description follows with abnormalities noted in 2 accessions.

Persistent chalazal megaspores enlarged greatly, attaining a volume approximately equal to that previously occupied by all 3 meiotic products, Figure 39. A series of 3 mitotic divisions in the persistent megaspore produced first a 2 nucleate, then a 4 nucleate, and finally an 8 nucleate embryo sac, Figures 40, 31 and 41, respectively.

Differentiation of 8 nucleate embryo sacs into normal mature megagametophytes included the formation of an egg apparatus, a central cell with 2 polar nuclei, and proliferation of 3 nuclei at the chalazal end of the sac with their inclusion into 6 - 9 antipodals. The egg apparatus consisted of a single egg cell, Figure 42, and 2 synergids, Figure 43.

A few enlarging megaspores of FL 640 grew completely through the epidermal layer of the nucellus and extended into a small cavity enclosed by the inner integuments, Figure 44.

Abnormal embryo sacs were found in 3 accessions. One post-anthesis ovule of D. decumbens, FL 594, contained a large embryo sac with a single large nucleus. Fourteen of 35 ovaries from FL 640 contained embryo sacs which extended out of the nucellus, through the micropyle into a large

cavity between the integuments and the ovary wall, Figure 45. Abnormal embryo sacs occurred in 13 of 29 non-aborted ovaries from the hybrid X125-1.

Four X125-1 ovaries contained ovules with more than the normal complement of gametophytic structures. Serial sections of an ovule with 2 egg cells, 2 synergids, 2 polar nuclei and several antipodals are shown in Figures 46, 47, and 48, respectively. It was impossible to determine if both egg cells were within the same embryo sac. Two additional ovules contained 2 egg cells, 1 with 2 pairs of polar nuclei and the other with 4 pairs. A fourth ovule contained 1 egg cell, 2 synergids, 3 polar nuclei and several antipodals.

One ovule from X125-1 contained an embryo sac with no egg apparatus, 1 large nucleus in the position normally occupied by the polar nuclei, and several antipodals, Figure 49.

Aberrant multicellular structures were found in 8 ovules from the hybrid X125-1. These structures contained as many as 6 cells and differed from normal megagametophytes in not containing recognizable egg cells, central cells, or antipodals, Figure 50.

The frequency of ovule abortion was scored for each of the 6 accessions and appears in Table 7. Ovaries scored were mature or had at least reached the 8 nucleate stage of embryo sac development.

Fertilization

Fertilization failed to occur in all but 1 of 84 D. pentzii, FL 645, pistils following pollination by D. pentzii, FL 640. Sixteen pistils were incubated for 15 minutes, 24 for 30 minutes, 15 for 45 minutes, and 29 for 60 minutes. The single ovary showing cytological evidence

Table 7. Frequency of ovule abortion.

Accession Number	Chromosome Number	Number of Ovules Examined	Number of Ovules Aborted	Percent Abortion
FL 615	18	59	0	0.00
FL 594	27	66	60	90.91
FL 640	36	35	3	8.57
FL 645	37	118	14	11.86
X125-1	45	41	12	29.27
FL 525	54	33	2	6.67

of fertilization was incubated for 30 minutes following pollination. Evidence of fertilization consisted of 2 nucleoli in the egg nucleus, Figure 51, and 2 nucleoli in the lower polar nucleus.

Two of 66 ovaries from D. decumbens, FL 594, showed evidence of fertilization after open pollination in the introduction nursery. One ovule contained a fertilized egg and 8 endosperm nuclei. A second ovule contained a 3 celled embryo and approximately 50 endosperm nuclei. The time between pollination and collection was unknown.

Figs. 1-51. All figures are photomicrographs of reproductive structures from 6 accessions of Digitaria. In figure legends, pollen mother cell is abbreviated PMC, and megaspore mother cell as MMC.

Fig. 1. Nine bivalents at metaphase I in a PMC of D. swazilandensis, FL 615. X1500.

Fig. 2. Eighteen univalents at anaphase I in a PMC of D. swazilandensis, FL 615. X750

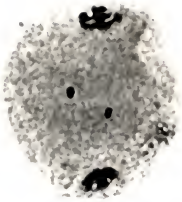

Fig. 3. Two lagging univalents at telophase I in a PMC of D. swazilandensis, FL 615. X1000.

Fig. 4. A configuration of 911 + 91 at metaphase I in a PMC of D. decumbens, FL 594. X700.

Fig. 5. Twenty-seven univalents at anaphase I in a PMC of D. decumbens, FL 594. X1000.

Fig. 6. Three dividing univalents at anaphase I in a PMC of D. decumbens, FL 594. X1200.

Fig. 7. A chromosome distribution of 12-15 at telophase I in a PMC of D. decumbens, FL 594. X1200.

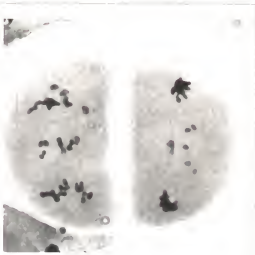

Fig. 8. Lagging chromosomes at anaphase and telophase II in secondary microsporocytes of D. decumbens, FL 594 X1200.

Fig. 9. A configuration of 18II + II at metaphase I in a PMC of D. pentzii, FL 645. X1500.

Fig. 10. A configuration of 16II + IIV + II at metaphase I in a PMC of D. pentzii, FL 645. X1200.

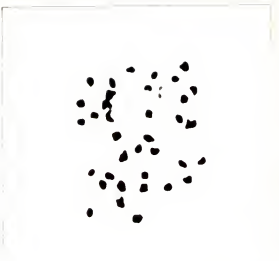

Fig. 11. Thirty-seven univalents at anaphase I in a PMC of D. pentzii, FL 645. X1500.

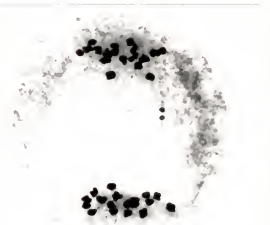

Fig. 12. A chromosome distribution of 18-18 plus 1 laggard at telophase I in a PMC of D. pentzii, FL 645. X1200.

Fig. 13. A divided univalent at telophase I in a PMC of D. pentzii, FL 645. X1200.

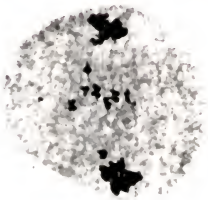

Fig. 14. Eight lagging univalents at telophase I in a PMC of D. pentzii, FL 645. X1200.

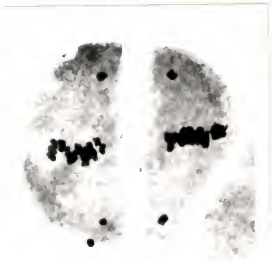

Fig. 15. Excluded chromosomes at metaphase II in secondary microsporocytes of D. pentzii, FL 645. X1200.

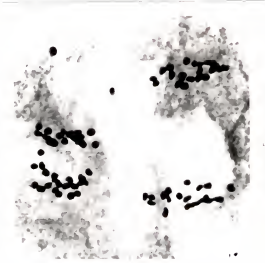

Fig. 16. Excluded chromosomes at anaphase II in secondary microsporocytes of D. pentzii, FL 645. X1200.

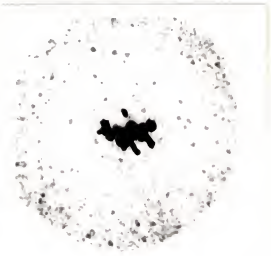

Fig. 17. Overlapping chromosomes at metaphase I in a PMC of X125-1. X1200.

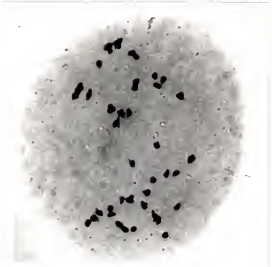

Fig. 18. Forty-five univalents at anaphase I in a PMC of X125-1. X1200.

Fig. 19. Four lagging univalents at anaphase I in a PMC of X125-1. X1200.

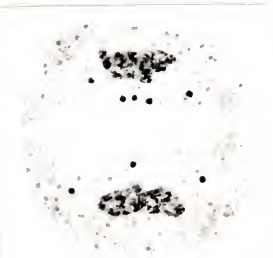

Fig. 20. Eight excluded chromosomes at prophase II in secondary microsporocytes of X125-1. X1200.

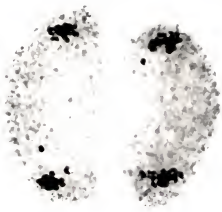

Fig. 21. Lagging chromosomes at telophase II in secondary microsporocytes of X125-1. X1000.

Fig. 22. Overlapping chromosomes at metaphase I in a PMC of FL 525. X1200.

Fig. 23. Late anaphase I in a PMC of D. sp., FL 525. X1200.

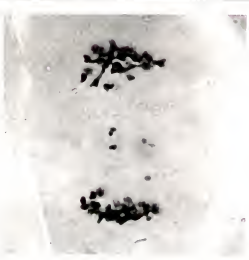

Fig. 24. Two lagging chromosomes at anaphase I in a PMC of D. sp., FL 525. X1200.

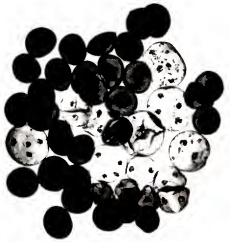

Fig. 25. Cells from a single anther of D. sp., FL 525, showing asynchrony of meiosis. X250.

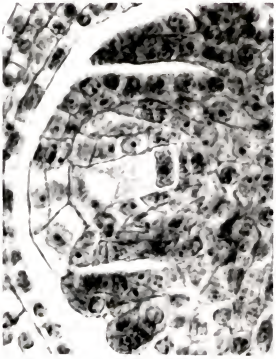

Fig. 26. A MMC at prophase I in an ovule of D. sp., FL 525. X750.

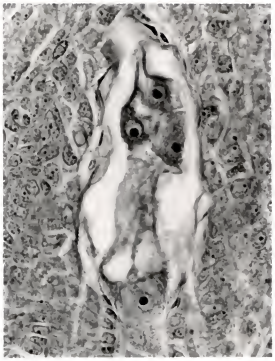

Fig. 27. An egg cell and 3 antipodals in an embryo sac of D. pentzii, FL 645. X750. Figures 27 and 28 show serial sections of the same embryo sac.

Fig. 28. Two synergids, 2 polar nuclei, and 3 antipodals in an embryo sac of D. pentzii, FL 645. X750. Figures 27 and 28 show serial sections of the same embryo sac.

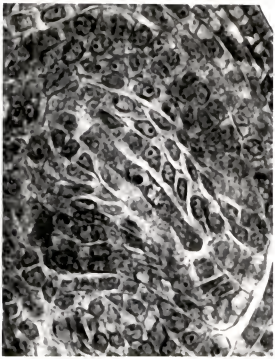

Fig. 29. A MMC with a centrally located nucleus in an ovule of X125-1. X625.

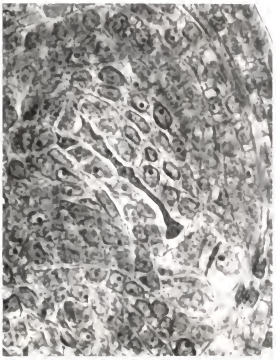

Fig. 30. A degenerating MMC in an ovule of X125-1. X625.

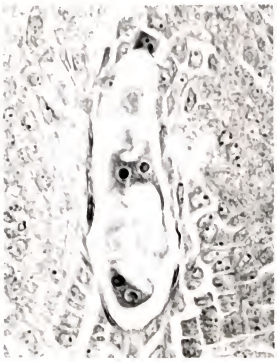

Fig. 31. A 4 nucleate embryo sac and a hypertrophied nucellar cell in an ovule of D. pentzii, FL 645. X1200.

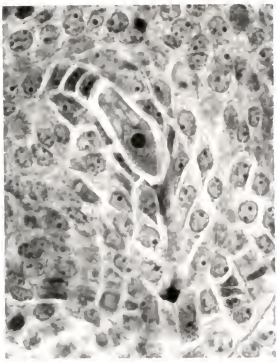

Fig. 32. A persistent megaspore and 3 hypertrophied nucellar cells in an ovule of D. pentzii, FL 645. X1000.

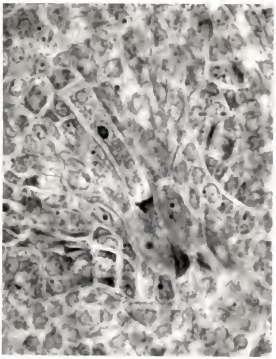

Fig. 33. A dyad of secondary megasporocytes in an ovule of D. pentzii, FL 640. X750.

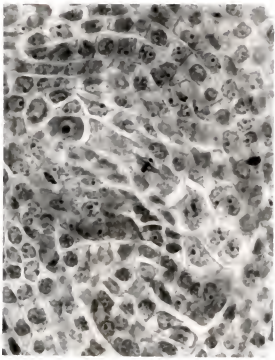

Fig. 34. Metaphase II in a secondary megasporocyte in an ovule of D. pentzii, FL 645. X625.

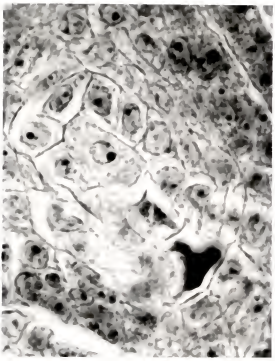

Fig. 35. A persistent megaspore, a degenerating megaspore, and a degenerating secondary megasporocyte in an ovule of D. sp., FL 525. X1000.

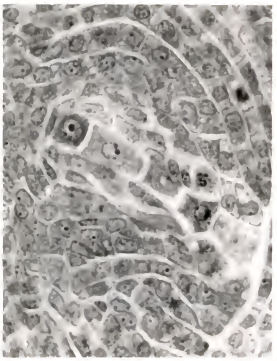

Fig. 36. A hypertrophied nucellar cell, a persistent megaspore, a degenerating megaspore, and a degenerating secondary megasporocyte in an ovule of D. pentzii, FL 645. X750.

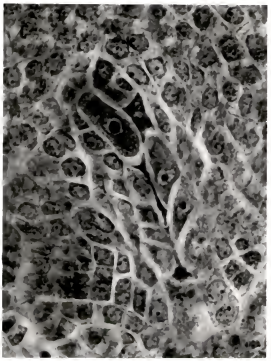

Fig. 37. A persistent megaspore in an ovule of D. pentzii, FL 645. X750.

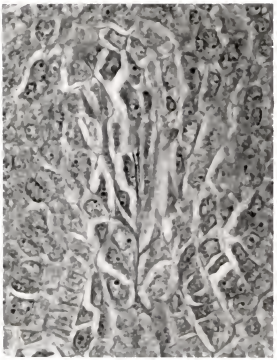

Fig. 38. All megaspores degenerating in an ovule of D. pentzii, FL 645. X1000.

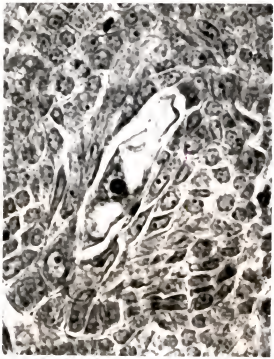

Fig. 39. The functional megaspore in an ovule of D. pentzii, FL 640. X750.

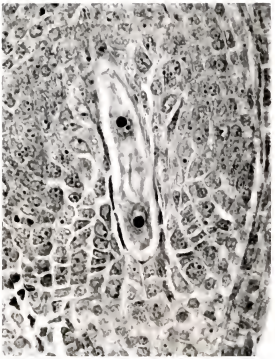

Fig. 40. A 2 nucleate embryo sac in an ovule of D. pentzii, FL 645. X750.

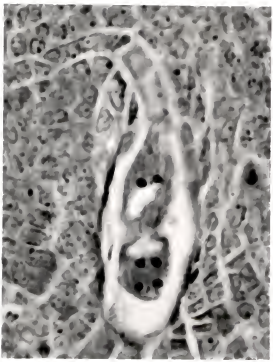

Fig. 41. An 8 nucleate embryo sac in an ovule from D. pentzii, FL 645. X625.

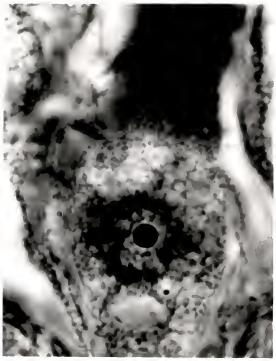

Fig. 42. An egg cell and 2 polar nuclei in an embryo sac of D. swazilandensis, FL 615. X1250.

Fig. 43. Two synergids and 1 polar nucleus in an embryo sac of D. swazilandensis, FL 615. X1250.

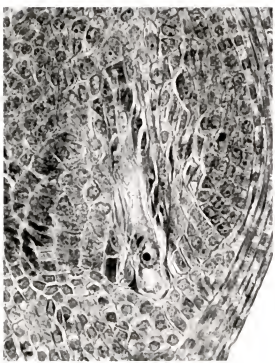

Fig. 44. A persistent megaspore of D. pentzii, FL 640, extended through the nucellar epidermis and in contact with an inner integument. X1000.

Fig. 45. An extra-ovular embryo sac in an ovary of D. pentzii, FL 640. X300.

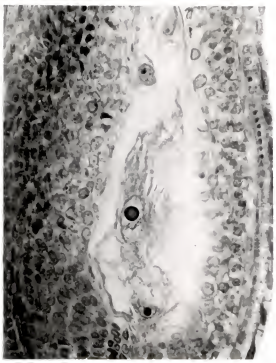

Fig. 46. An egg cell, 1 polar nucleus, and 2 antipodals in an ovule of X125-1. X750. Figures 46, 47, and 48 show serial sections of the same ovule.

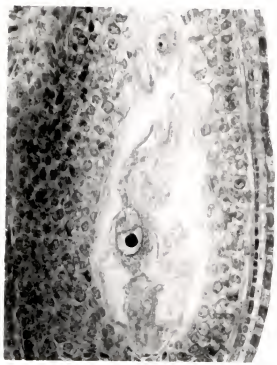

Fig. 47. Degenerating synergids, 1 polar nucleus, and 2 antipodals in an ovule of X125-1. X750. Figures 46, 47, and 48 show serial sections of the same ovule.

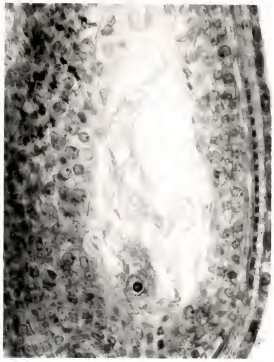

Fig. 48. An egg cell in an ovule of X125-1. X750. Figures 46, 47, and 48 show serial sections of the same ovule.

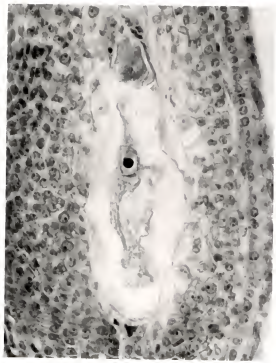

Fig. 49. One large nucleus and several antipodals in an embryo sac of X125-1. X750.

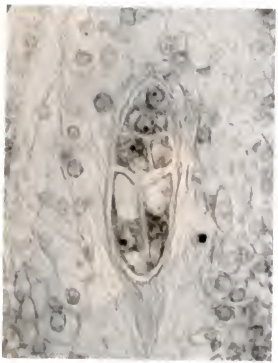

Fig. 50. An aberrant multicellular structure in an ovule of X125-1. X1000.

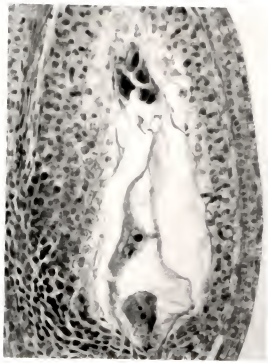

Fig. 51. An embryo sac of D. pentzii, FL 645, killed 30 minutes after pollination with D. pentzii, FL 640. X300.

DISCUSSION

The Digitaria accessions studied varied considerably in their reproductive behavior. Irregularities were found during microsporogenesis, megasporogenesis, and embryo sac development in 5 of the 6 accessions. The reproductive characteristics of each accession are summarized in Table 8.

D. swazilandensis, FL 615

Regular meiotic chromosome behavior during microsporogenesis and a high frequency of apparently normal embryo sacs in the diploid D. swazilandensis, FL 615, indicated that low seed set obtained with this accession (37) was not due to irregularities in gametogenesis. Low seed set may result from self incompatibility and incompatibility with the 4 other D. swazilandensis accessions in the introduction nursery.

D. decumbens, FL 594

Schank and Decker (37) found no seeds in open pollinated inflorescences of the triploid D. decumbens, FL 594. In the present study, only 6 of 66 post-anthesis ovaries from open pollinated inflorescences contained embryo sacs, and two had been fertilized. Low seed set was probably due to lethal genetic imbalance in gametophytes resulting from meiotic irregularities.

D. pentzii, FL 640

Meiotic chromosome behavior was regular in more than 86 percent of the pollen mother cells of the tetraploid D. pentzii, FL 640. However,

Table 8. Reproductive characteristics of 6 Digitaria accessions.

Accession Number	Chromosome Number	Pollen Stainability	Mean Number of Lagging Chromosomes	Percent Ovule Abortion	Percent Seed Set
FL 615	18	78.0	0.004	0.00	1[a]
FL 594	27	11.5	3.081	90.91	0[a]
FL 640	36	68.0	0.132	8.57	10[a]
FL 645	37	82.0	1.432	11.86	50[a]
X125-1	45	12.0	2.935	29.27	0 - 10[b]
FL 525	54	76.0	0.347	6.67	10[a]

[a]Schank and Decker (37).

[b]van Heemert and Schank (45).

nearly half of the ovaries examined contained embryo sacs which extended out of the ovule, and an additional 8.57 percent aborted before reaching maturity.

Extra-ovular embryo sac development was found in 1 of 55 D. valida ovaries, and 6 of 12 mature ovaries of Fingerhuthia africana Lehm in the tribe Chlorideae by Emery and Brown (17). In the single D. valida ovary with an extra-ovular embryo sac, the sac had grown obliquely through the nucellus and into the ovary wall in the region where the ovule was attached to the ovary. In ovaries of F. africana, extra-ovular embryo sacs extended through the micropyle into the ovarian locule as was found in D. pentzii, FL 640. Fertilization and embryo development occurred in extra-ovular embryo sacs of F. africana. Extra-ovular embryo sacs of D. pentzii, FL 640, may also be capable of embryo development, but this remains uncertain since all ovaries examined were collected prior to anthesis. The low rate of seed set reported (37) in D. pentzii, FL 640, suggests that extra-ovular embryo sacs did not function in seed formation.

D. pentzii, FL 645

The high rate of pollen stainability and low rate of ovule abortion found in D. pentzii, FL 645, were unexpected since meiosis was irregular in this accession. More than half the pollen mother cells observed at metaphase I contained a quadrivalent, and the mean number of lagging chromosomes was 1.43 at anaphase and telophase I. Apparently normal embryo sacs were found in 88 percent of the ovaries examined. The high frequency of lagging chromosomes, stainable pollen and embryo sacs suggests the loss of chromosomes during gametogenesis is not lethal. The high rate of seed set (37) and successful in vitro pollen germination support this hypothesis.

Hybrid X125-1

A high rate of pollen and embryo sac abortion was found in the pentaploid hybrid X125-1, and was probably due to irregular chromosome distribution during gametogenesis. Only 12 percent of the pollen and 39 percent of the embryo sacs of this hybrid appeared normal, accounting for the low rate of seed set observed by van Heemert and Schank (45).

D. sp., FL 525

The low rate of seed set reported in the hexaploid FL 525 (37) can not be attributed entirely to irregularities in gametogenesis. Pollen stainability was 76.0 percent and only 6.67 percent embryo sac abortion was found.

SUMMARY

Studies on the reproductive behavior of 6 Digitarias indicated that seed set was low in 4 of the 6 accessions due to genetic imbalance in gametophytes resulting from meiotic irregularities.

A diploid accession of D. swazilandensis, FL 615, exhibited few irregularities in gametogenesis, but produced very few seed. Low seed set was probably due to self incompatibility.

Digitaria decumbens, FL 594, was triploid and showed highly irregular chromosome behavior during microsporogenesis. Unlike triploid pangolagrass (D. decumbens, P. I. 111110), this accession produced a few functional embryo sacs and seed.

Irregular meiosis during microsporogenesis, and ovule abortion, contributed to the low rate of seed set found in D. pentzii, FL 640. In some ovaries of this accession, embryo sacs protruded through the micropyle into the ovarian locule.

Aneuploidy and irregular chromosome behavior were found in D. pentzii, FL 645. One quadrivalent was found in more than half the pollen mother cells observed at metaphase I, and 37 univalents were seen at anaphase I. Viable pollen and mature embryo sacs were produced in spite of the irregularities noted in this accession. No evidence of apomixis was found, and seed produced by this accession was probably the result of normal sexual reproductive processes.

The pentaploid hybrid, X125-1, exhibited highly irregular meiosis during microsporogenesis, but produced some stainable pollen. Embryo

sac development was normal in 39 percent of the ovaries examined. Seed production was probably the result of normal sexual reproduction. The presence of 2 egg cells in a few X125-1 ovules indicated that twin seedlings might develop from some seeds of this hybrid.

Meiotic chromosomes in pollen mother cells of D. sp., FL 525, failed to condense as thoroughly as those of the other accessions, and were difficult to analyze. Asychrony of meiotic stages was found among pollen mother cells in the same locule of some anthers. Pollen stainability and ovule examination, however, indicated a high rate of normal gametophyte formation, and did not account for the low rate of seed set observed with this accession.

Cytological examination of the reproductive processes of the 6 Digitarias indicated that while meiotic irregularities greatly reduce seed set in some accessions, none were completely sterile. All accessions studied could be used in a hybridization program if a procedure for recovering seeds which occur rarely was found.

LITERATURE CITED

1. Boyd, F. T. and V. G. Perry. 1969. Sting nematodes attack forage grasses in Florida. Sunshine State Agr. Res. Rept. 14:3-6.

2. Brown, W. V. 1948. A cytological study in the Gramineae. Am. J. Botan. 35:382-395.

3. Brown, W. V. and W. H. P. Emery. 1958. Apomixis in the Gramineae: Panicoideae. Am. J. Botan. 45:253-263.

4. Burton, G. W. 1942. A cytological study of some species in the tribe Paniceae. Am. J. Botan. 29:355-359.

5. Carmichael, J. W., W. B. Sherman, and S. C. Schank. 1970. A rapid stain for plant cell nuclei. Hort. Science 5:116-117.

6. Chandrasekharan, P. 1961. Cytological studies on some South Indian grasses. (Abstr.) Plnt. Breed. Abstr. 32:788.

7. Chen, C. C. and C. C. Hsu. 1961. Cytological studies on Taiwan grasses. I. Tribe Paniceae. Botan. Bull. Acad. Sinica 2: 101-110.

8. Chippendall, Lucy K. A. 1955. A guide to the identification of grasses in South Africa, p. 1-527. In D. Meredith, (ed.), The grasses and pastures of South Africa. Cape Times Ltd., Union of South Africa.

9. Church, G. L. 1929. Meiotic phenomena in certain Gramineae. II. Paniceae and Andropogoneae. Botan. Gaz. 88:63-84.

10. Covas, G. and B. Schnack. 1947. Estudios cariologicos en autofitas. Rev. Argentina Agron. 14:224-231.

11. Darlington, C. D. and A. P. Wylie. 1956. Chromosome atlas of flowering plants. The Macmillan Co., New York. 519 p.

12. de Wet, J. K. J. 1954. Chromosome numbers of a few South African grasses. Cytologia 19:103.

13. de Wet, J. K. J. 1958. Additional chromosome numbers in Transvaal grasses. Cytologia 23:113-118.

14. de Wet, J. K. J. 1960. Chromosome numbers and some morphological attributes of various South African grasses. Am. J. Botan. 47:44-49.

15. de Wet, J. K. J. and L. J. Anderson. 1956. Chromosome numbers in Transvaal grasses. Cytologia 21:1-10.

16. Dirven, J. G. P. and H. A. van Hoof. 1960. A destructive virus disease of pangolagrass. T. Pl. Ziekten 66:344-349.

17. Emery, W. H. P. and W. V. Brown. 1957. Extra-ovular development of embryos in two grass species. Bull. Torrey Botan. Clb. 84:361-365.

18. Gould, F. W. 1960. Chromosome numbers in southwestern grasses. II. Am. J. Botan. 47:873-877.

19. Gould, F. W. 1963. Cytotaxonomy of Digitaria sanguinalis and D. adscendens. Brittonia 15:241-244.

20. Henrard, J. Th. 1950. Monograph of the genus Digitaria. Universitaire Pers., Leiden, The Netherlands. 999 p.

21. Hirayoshi, I. and T. Yasue. 1955. Cytogenetical studies on forage plants. II. Chromosome numbers and some specific characters in Japanese native species of Digitaria. (Abstr.) Plnt. Breed. Abstr. 26:232.

22. Hunter, A. W. S. 1934. A karyosystematic investigation in the Gramineae. Canad. J. Res. 11:213-241.

23. Jensen, W. A. 1962. Botanical histochemistry. W. H. Freeman and Co., San Francisco, California. 408 p.

24. Johansen, D. A. 1940. Plant microtechnique. McGraw-Hill Book Co., New York, New York. 523 p.

25. McCaleb, J. E. and E. M. Hodges. 1969. Slenderstem digitgrass. Fla. Agr. Exp. Sta. Circ. S-201. 11 p.

26. Moffett, A. A. and R. Hurcombe. 1949. Chromosome numbers in South African grasses. Heredity 3:369-373.

27. Nestel, B. L. and M. J. Creek. 1962. Pangola grass. Herb. Abstr. 32:1-7.

28. Oakes, A. J. 1965. Digitaria collection from South Africa. Trop. Agr., Trin. 42:323-331.

29. Pritchard, A. J. and K. F. Gould. 1964. Chromosome numbers in some introduced and indigenous legumes and grasses. CSIRO Aust. Div. Trop. Past. Tech. Paper No. 2. pp. 2-18.

30. Purcell, C. M. 1965. Embryo-sac development in two accessions of giant pangola Digitaria valida Stent. J. Agr. Univ. Puerto Rico 49:477-483.

31. Raman, V. S., P. Chandrasekharan, and D. Krishnaswami. 1959. Chromosome numbers in Gramineae. Curr. Sci. 28:453-454.

32. Rotar, P. P. and U. Urata. 1966. A hexaploid pangolagrass clone. Crop Sci. 6:218-219.

33. Sass, J. E. 1958. Botanical microtechnique. 4th ed. The Iowa State Press, Ames, Iowa. 228 p.

34. Schank, S. C. 1963. Improving pangolagrass. Sunshine State Agr. Res. Rept. 8:14-15.

35. Schank, S. C., J. W. Carmichael, and R. L. Stanley, Jr. 1970. Hybridization of Digitarias having winter hardiness. Proc. Soil Crop Sci. Soc. Fla. (in press).

36. Schank, S. C. and H. F. Decker. 1965. The Florida garden of Digitaria introductions. Sunshine State Agr. Res. Rept. 10:8-9.

37. Schank, S. C. and H. F. Decker. 1967. Cytological investigations in the genus Digitaria. Proc. Soil Crop Sci. Soc. Fla. 27: 96-101.

38. Schank, S. C., H. F. Decker, G. B. Killinger, and R. J. Allen, Jr. 1966. Agronomic and cytotaxonomic comparisons between Digitaria decumbens and D. pentzii. Crop Sci. 6:82-83.

39. Schank, S. C. and J. R. Edwardson. 1968. Cytological examination of pangolagrass (Digitaria decumbens Stent) infected with stunt virus. Crop Sci. 8:118-119.

40. Shambulingappa, K. G. 1967. Studies on pachytene chromosomes in the genus Digitaria. Genetica 38:381-387.

41. Shambulingappa, K. G. 1969. Cytomorphological and sterility studies in Digitaria polevansii Stent. J. Agr. Univ. Puerto Rico 53:106-112.

42. Sheth, A. A., Lillian Yu, and J. R. Edwardson. 1956. Sterility in pangolagrass (Digitaria decumbens Stent). Agron. J. 48: 505-507.

43. Sotomayor-Rios, A., K. F. Schertz, R. Woodbury, and J. Velez-Fortuno. 1960. Taxonomic description and reproductive behavior of giant pangola (Digitaria valida Stent). J. Agr. Univ. Puerto Rico 44:53-59.

44. Tateoka, T. 1956. The chromosomes of Digitaria adscendens. (Abstr.) Plnt. Breed. Abstr. 26:71-72.

45. van Heemert, C. and S. C. Schank. 1968. New breeding techniques: Terylene bags for Digitaria breeding. Proc. Soil Crop Sci. Soc. Fla. 28:150-155.

46. Vega-Luna, M., S. C. Schank, and O. C. Ruelke. 1967. Seed set - temperature relationships as measured in a Digitaria milanjiana cross. Proc. Soil Crop Sci. Soc. Fla. 27:101-106.

47. Virkki, N. and C. M. Purcell. 1967. Observations on the behavior, genetics and cytology of two South African Digitaria valida Stent accessions in Puerto Rico. J. Agr. Univ. Puerto Rico 51:269-285.

48. Wilms, H. J., J. W. Carmichael, and S. C. Schank. 1970. Cytological and morphological investigations on the grass Hemarthria altissima (Poir) Stapf et C. E. Hubb. Crop Sci. 10: 309-312.

49. Yasue, T. 1957. Studies on hybrids in Digitaria. II. The F_1 plants, D. ciliaris Pers. X D. ischaemum Muhl. (In Japanese, English Summary); Jap. J. Breed. 6:64-67.

50. Young, E. M. and C. G. Crocker. 1933. Chromosome studies in Digitaria species. J. South Afr. Sci. 30:258-265.

BIOGRAPHICAL SKETCH

John W. Carmichael was born April 21, 1942, in Miami, Florida. He attended primary and secondary schools in Miami, and was graduated from Southwest Miami High School in June, 1960. In September, 1960, he enrolled at the University of Florida, where he received the degree of Bachelor of Science in Agriculture in April, 1965. From June to December, 1965, he was employed by the Botany Department as a herbarium assistant. He entered graduate school at the University of Florida in January, 1966, and majored in Agronomy. He held graduate research and teaching assistantships from January, 1965, until he completed the requirements for the degree of Doctor of Philosophy, in December, 1970.

John W. Carmichael is married to the former Elaine Arrington, and is the father of two children. He is a member of Gamma Sigma Delta and Phi Sigma Biological Society.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Stanley C. Schank

Stanley C. Schank, Chairman
Associate Professor of Agronomy

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Mildred M. Griffith

Mildred M. Griffith
Professor of Botany

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Frederick T. Boyd

Frederick T. Boyd
Professor of Agronomy

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Wayne B. Sherman

Wayne B. Sherman
Assistant Professor of Fruit Crops

This dissertation was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December, 1970

Dean, College of Agriculture

Dean, Graduate School